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Subverting sterols: rerouting an oxysterol-signaling pathway to promote tumor growth

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Oxysterols are oxidized derivatives of cholesterol that are generated enzymatically or through autoxidation. Initially identified as important lipid signaling molecules in the context of atherosclerosis and inflammation, accumulated evidence indicates that these lipid-signaling molecules can have pleiotropic effects on the fate and function of the immune system. These effects range from the regulation of immune cell survival and proliferation to chemotaxis and antiviral immunity. New studies now indicate that tumor-derived oxysterols can serve to subvert the immune system by recruiting protumorigenic neutrophils into the tumor microenvironment. The consequence of this recruitment is the generation of proangiogenic factors and matrix metalloproteinase proteins that provide a tumor a significant growth and survival advantage. In combination with other recent studies, these data highlight the ongoing cross talk between sterol metabolism and the immune system, and they raise the intriguing possibility that targeting oxysterol pathways could serve as a novel therapeutic approach in the war on cancer.

Cholesterol is a lipid that plays essential and diverse roles in biology. In vertebrates, cholesterol can be synthesized through a series of enzymatic steps in the endoplasmic reticulum of a cell or can be taken up from the serum as cholesterol esters stored in lipoprotein particles (Voet and Voet, 2011). Cellular cholesterol is predominately found in plasma membranes, where it serves as an important regulator of membrane fluidity and dynamics. Additional important functions include serving as the backbone of steroid hormone and bile acid synthesis, and lipid modification to proteins. Oxysterols are oxidized derivatives of cholesterol that are generated through the enzymatic actions of P450 cytochrome oxidases (e.g., CYP7b1) or via autoxidation (Russell, 2000). Although oxysterols exist in very small quantities within the cell when compared with cholesterol, they have potent and pleiotropic biological effects. The best-defined example of the cross talk between oxysterols and innate immunity has been described in the pathogenesis of atherosclerosis and related cardiovascular disease pathogenesis, and we direct the reader to several excellent reviews on this subject (Bensinger and Tontonoz, 2008; Moore and Tabas, 2011; Calkin and Tontonoz, 2012). Unexpectedly, several recent studies have identified oxysterols as critical signaling molecules in the development and function of the immune system (Bensinger et al., 2008; A-Gonzalez et al., 2009; Hannedouche et al., 2011; Liu et al., 2011; Hong et al., 2012; A-Gonzalez et al., 2013; Westerterp et al., 2013). In this issue, a new study by Raccosta et al. highlights how tumor-derived oxysterols are potent signaling molecules co-opted by solid tumors to recruit protumorigenic neutrophils into the tumor microenvironment. These studies add to the growing list of how oxysterols modulate the immune system and raise the intriguing possibility that targeting tumor-derived oxysterols could serve as a fresh approach to controlling solid tumor growth.

The best understood function of oxysterol signaling is in the regulation of cellular cholesterol homeostasis. Cellular cholesterol levels are largely maintained through the activity of two transcription factors, the sterol regulatory element binding proteins (SREBP1 and SREBP2) and the liver X receptors (LXRα and β; Horton et al., 2002; Bensinger and Tontonoz, 2008). If a cell has sufficient cholesterol, then SREBP proteins are held inactive in the endoplasmic reticulum via its association with two sterol-sensing proteins, INSIG and SCAP. Under conditions of low cellular cholesterol or specific oxysterols (e.g., 25-HC), SREBP proteins translocate to the nucleus and transactivate the genes that encode the enzymes required for synthesis of cholesterol, as well as for proteins involved in low-density lipoprotein uptake (e.g., LDL receptor; Horton et al., 2002). Conversely, when excess oxysterols (e.g., 22-(R) and 25-hydroxycholesterol) accumulate in the cell, they can directly bind to and activate LXRs, resulting in transcriptional up-regulation of genes involved in cholesterol efflux (Calkin and Tontonoz, 2012). LXR proteins also attenuate inflammation in immune cells through their ability to directly repress inflammatory gene expression through a process termed transrepression (Bensinger and Tontonoz, 2008). In combination, these two transcriptional programs regulate a cell's cholesterol levels while also avoiding deleterious inflammation associated with excess lipid accumulation in tissues.

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Oxysterols beckon cells

Although the LXR pathway plays an important role in regulating the cross talk between lipid metabolism and immunity, it is clear that not all oxysterols bind to and activate LXRs. Moreover, the addition of oxysterols to cultures can have pleiotropic effects on immune cells in an LXR-independent manner. Thus, it has been of considerable interest to the field to unravel the molecular mechanisms by which oxysterols influence host immunity. A major advance in our understanding comes from recent exciting studies that demonstrated that oxysterols bind to and directly activate G protein–coupled receptors (GPCRs) on immune cells. The type I IFN inducible oxysterol 7α,25-OHC was identified as the ligand for the orphan receptor Epstein-Barr virus–induced gene 2 (Ebi2; Hannedouche et al., 2011; Liu et al., 2011). This oxysterol, and other closely related oxysterol species, induce the migration of EBI2 receptor–positive B and T lymphocytes and DCs. The generation of the most potent oxysterol 7α,25-OHC appears to be dependent on two enzymes, 25-hydroxycholesterol 7α-hydroxylase (Cyp7b1) and cholesterol 25-hydroxylase (Ch25h). LPS treatment of mice induced significant levels of 7α,25-OHC and migration of immune cells within the spleen in a Ch25h-dependent manner. Intriguingly, Ch25h has long been associated with type I IFN responses, and recent work indicates that one of the products of this enzyme, 25-hydroxycholesterol (25-HC), can have potent antiviral activity, although the mechanism of action remains poorly described (Blanc et al., 2013; Liu et al., 2013). Nevertheless, these studies clearly demonstrate that multiple oxysterols are generated in the context of normal immune responses and their signals have important pleiotropic effects on host immunity.

Subverting oxysterols signaling to a tumors advantage

Tumors are expert at co-opting inflammatory pathways, and dampening host immunity to their growth and survival advantage (Hanahan and Weinberg, 2011). Given the emerging importance of oxysterols in facilitating host immunity, it is perhaps not surprising that tumors might subvert oxysterol pathways to dampen host antitumor immunity. Indeed, studies have revealed a critical role for oxysterols in controlling the expression pattern of the chemokine (C-C motif) receptor 7 (CCR7) on tumor infiltrating DCs (Villablanca et al., 2010). Tumor-derived oxysterols were found to down-regulate the chemokine (C-C motif) receptor 7 (CCR7) in an LXR-dependent manner. CCR7 is a member of the GPCR family, which was initially identified as a gene up-regulated in EBV infection, and plays a critical role in driving DC migration to draining lymph nodes. As such, suppression of CCR7 would effectively trap DCs in the tumor, thereby interfering with antigen presentation to antitumor T cells and host antitumor immunity. The mechanism of oxysterol-mediated CCR7 down-regulation was dependent on activation of LXRα in DCs. In light of the recent data showing that oxysterols act as chemoattractants, it would be of interest to test oxysterols identified in tumor supernatants for chemotactic ability on DCs independent of LXR signaling.

An additional layer of complexity is uncovered by new studies in this issue by Raccosta et al. (Fig. 1). Using mass spectrometry, they found that tumors produce an array of hydroxycholesterol species, in particular 22-HC and 27-HC, in sufficient quantities to activate LXR in tumor tissue as well as at distant tissues sites of the host, such as BM. Interestingly, one functional consequence of oxysterol generation by cancer cells was a marked increase in the number of $CD11b^{high}Gr1^{high}$ neutrophils infiltrating the tumor stroma (Fig. 1 A). Using a series of elegant approaches, including parabiosis of congenic hosts, they establish that oxysterols generated by tumor cells are necessary for the continuous recruitment of neutrophils to the tumor site. This recruitment appeared to be dependent on the binding of 22-HC oxysterols to the GPCR CXCR2 and was independent of LXR activity. Importantly, inactivation of oxysterol generation by tumors via the ectopic expression of the cholesterol sulfotransferase 2B1b (SULT2B1b) significantly attenuated neutrophil infiltrate and tumor growth.

Although one might expect that infiltration of neutrophils into tumors would favor a proinflammatory environment and facilitate antitumor host immunity, it has become increasingly clear that in solid tumors, invading neutrophils (or other myeloid cells) provide an important growth and survival advantage for tumors (Motz and Coukos, 2011). Consistent with this concept, high levels of tumor-infiltrating neutrophils and CXCR2 expression in tumor stroma are associated with poorer clinical outcomes in some human cancers (Jensen et al., 2009; Saintigny et al., 2013; Singh et al., 2013). The mechanisms underlying the protumorigenic function of tumor-associated neutrophils are likely multifactorial, but the Raccosta et al. study suggests that it is reliant in part on the production of the proangiogenic factor BV8 and the release of matrix metalloproteinase MMP-9. Interestingly, the authors did not observe a significant suppressive function of tumor-associated neutrophils on T cell populations, distinguishing their protumorigenic role from that of the immune modulatory activity attributed to many other infiltrating myeloid cells, such as myeloid-derived suppressor cells.

Finally, these findings shed light as to why tumors might produce such significant levels of oxysterols. These molecules can have significant antiproliferative effects, and it would seem that localized production of oxysterols would attenuate cancer cell cycle progression and survival. However, these data indicate that the chemotactic signals of oxysterols may outweigh the antiproliferative effects, and imply that inhibition of oxysterols could provide a novel therapeutic target for both the generation of efficient antitumor immune responses and the inhibition of growth-promoting myeloid tumor-infiltrating cells.

Like clockwork: oxysterols regulate innate immune cell homeostasis

Recent studies have also identified a prominent role for oxysterol signaling in the regulation of immune and hematopoietic cell homeostasis under

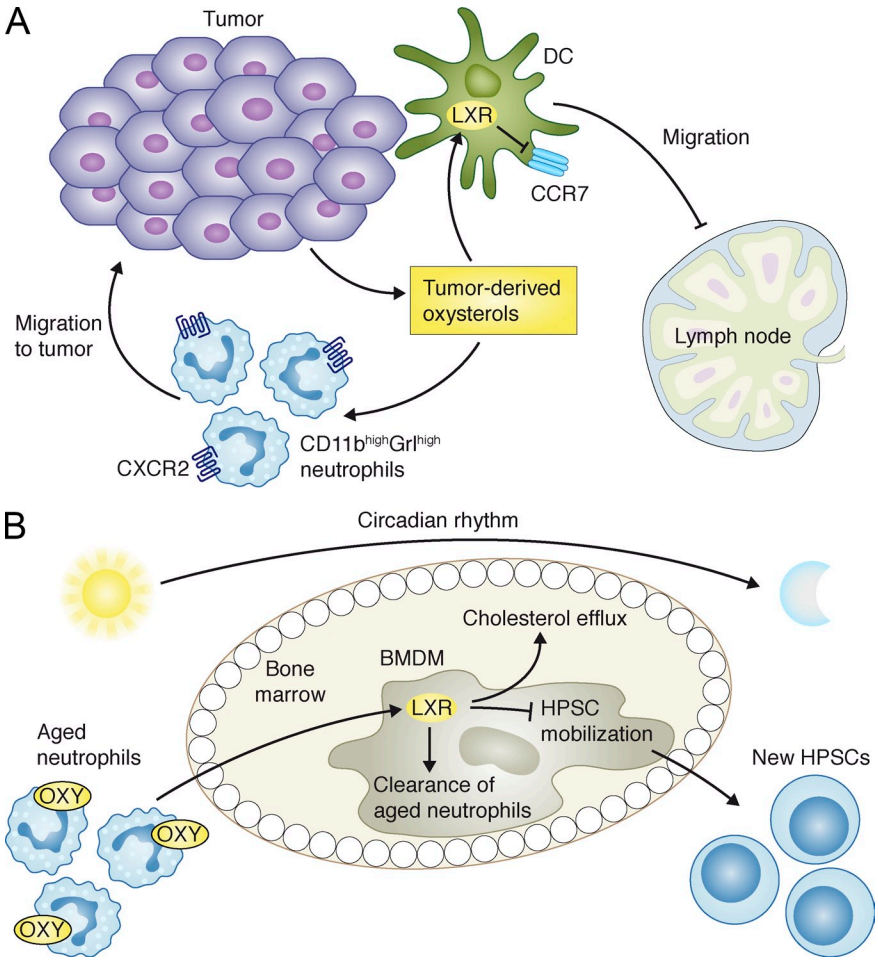

Figure 1. An integral role for oxysterol in neutrophil fate and function. (A) Tumor-derived oxysterols facilitate tumor growth and survival. Some oxysterols inhibit tumor-associated DCs from migrating to lymphoid organs in an LXR-dependent manner, thereby promoting tumor evasion of the immune system. In addition, tumor-secreted oxysterols can act as a chemoattractant for proangiogenic and protumorigenic CD11b^{high} GR-1^{high} neutrophils via the activation of GPCRs (e.g., CXCR2). (B) Aged neutrophils are phagocytized by BM macrophages in a circadian cycle. Phagocytosis activates LXR presumably by cholesterol and oxysterols in aged neutrophils. Activation of LXR restricts the size of the hematopoietic niche and inhibits mobilization of hematopoietic progenitor stem cells (HPSCs).

noninflammatory and nontumor conditions. Building on studies that identified LXR as an important mediator apoptotic cell clearance by macrophages (A-Gonzalez et al., 2009), it was suggested that LXR may play a physiological role in the regulation of neutrophil homeostasis (Hong et al., 2012). Neutrophils are the most abundant cell type of the immune system with a short half-life in healthy individuals, on the order of 3–12 h. Under noninflammatory conditions, neutrophils are not activated, and the vast majority of these cells are cleared by resident tissue macrophages in the liver, spleen, and BM. The molecular mechanisms underlying the clearance of these cells by tissue macrophages remain poorly understood. Mice lacking both isoforms of LXR were found to have increased numbers of neutrophils in blood, spleen, and liver under noninflammatory conditions. In contrast, pharmacologic activation of the LXR pathway decreased circulating neutrophils in mice and increased aged neutrophil clearance by macrophages. Mechanistic studies revealed that engulfed apoptotic neutrophils activate LXR, resulting in the up-regulation of the c-mer proto-oncogene tyrosine kinase (MERTK), a key receptor in apoptotic cell phagocytosis. Moreover, activation of LXR by apoptotic neutrophils in spleen repressed the expression of the granulopoietic cytokines IL-23 and IL-17 (Hong et al., 2012). How aged neutrophils find the appropriate resident tissue macrophages remains to be determined. Given the results of Raccosta et al. herein that oxysterols can drive neutrophil chemotaxis in a CXCR2-dependent manner, it would be of interest to determine if local production of oxysterols, such as 22-HC, by tissue and BM macrophage serves as the elusive "find me" signals delivered to aged neutrophils.

Intriguing new studies have provided further insights into the intricate nature of cross talk between hematopoietic progenitor cells (HPCs), neutrophils, and the LXR signaling pathway (Casanova-Acebes et al., 2013; Fig. 1 B). Using elegant imaging techniques in combination with parabiosis, this study established that rhythmic oscillations of peripheral neutrophil and HPCs are intimately linked through the function of LXR. The authors found that senescent neutrophils home to the BM in a circadian manner where they are presumably cleared by BM macrophage. The engulfment of neutrophils by BM macrophage reduced the hematopoietic niche and resulted in the subsequent release of HPCs into the peripheral circulation. The genetic ablation of LXR resulted in the attenuation of the hematopoietic niche oscillations and rhythmic release of HPCs, suggesting that oxysterols play a fundamental role in controlling hematopoiesis. Collectively, these studies indicate that that oxysterols play an important role in neutrophil biology.

LXR was also recently shown to be required for the differentiation of splenic marginal zone and metallophilic macrophage from hematopoietic precursors (A-Gonzalez et al., 2013). There is considerable heterogeneity in splenic macrophage populations, and little is known regarding the transcriptional program that drives the differentiation of tissue macrophage from myeloid precursors. It remains unclear why oxysterols and LXR signaling would control the differentiation of these specialized populations of macrophages. Nevertheless,

these findings, in combination with the studies discussed above, provide strong evidence that sterol signaling through the LXR axis plays a fundamental role in the control of hematopoietic cell fate and function.

A few final thoughts

Accumulating evidence indicates that oxysterols play an active and important role in the regulating the fate and function of immune cells. The study by Raccosta et al. presented herein demonstrates how this cross talk between lipid metabolism and the immune system can be subverted by tumors to promote their growth and survival. Although it is easy to hypothesize that oxysterols could serve as an important therapeutic target, it is important to realize that we are just beginning to understand how these lipid molecules impact inflammation, immunity, and hematopoiesis. Given the pleiotropic effect of these lipids, it will be necessary for the field to mechanistically determine when, where, and how these molecules are signaling. With this information in hand, it will become more apparent whether oxysterol pathways should be considered viable therapeutic targets.

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REFERENCES

A-Gonzalez, N., S.J. Bensinger, C. Hong, S. Beceiro, M.N. Bradley, N. Zelcer, J. Deniz, C. Ramirez, M. Díaz, G. Gallardo, et al. 2009. Apoptotic cells promote their own clearance and immune tolerance through activation of the nuclear receptor LXR. *Immunity*. 31:245–258. http://dx.doi.org/10.1016/j.immuni.2009.06.018

A-Gonzalez, N., J.A. Guillen, G. Gallardo, M. Diaz, J.V. de la Rosa, I.H. Hernandez, M. Casanova-Acebes, F. Lopez, C. Tabraue, S. Beceiro, et al. 2013. The nuclear receptor LXRα controls the functional specialization of splenic macrophages. *Nat. Immunol.* 14:831–839. http://dx.doi.org/10.1038/ni.2622

Bensinger, S.J., and P. Tontonoz. 2008. Integration of metabolism and inflammation by lipid-activated nuclear receptors. *Nature*. 454:470–477. http://dx.doi.org/10.1038/nature07202

Bensinger, S.J., M.N. Bradley, S.B. Joseph, N. Zelcer, E.M. Janssen, M.A. Hausner, R. Shih, J.S. Parks, P.A. Edwards, B.D. Jamieson, and P. Tontonoz. 2008. LXR signaling couples sterol metabolism to proliferation in the acquired immune response. *Cell*. 134:97–111. http://dx.doi.org/10.1016/j.cell.2008.04.052

Blanc, M., W.Y. Hsieh, K.A. Robertson, K.A. Kropp, T. Forster, G. Shui, P. Lacaze, S. Watterson, S.J. Griffiths, N.J. Spann, et al. 2013. The transcription factor STAT-1 couples macrophage synthesis of 25-hydroxycholesterol to the interferon antiviral response. *Immunity*. 38:106–118. http://dx.doi.org/10.1016/j.immuni.2012.11.004

Calkin, A.C., and P. Tontonoz. 2012. Transcriptional integration of metabolism by the nuclear sterol-activated receptors LXR and FXR. *Nat. Rev. Mol. Cell Biol.* 13:213–224.

Casanova-Acebes, M., C. Pitaval, L.A. Weiss, C. Nombela-Arrieta, R. Chèvre, N.A-González, Y. Kunisaki, D. Zhang, N. van Rooijen, L.E. Silberstein, et al. 2013. Rhythmic modulation of the hematopoietic niche through neutrophil clearance. *Cell*. 153:1025–1035. http://dx.doi.org/10.1016/j.cell.2013.04.040

Hanahan, D., and R.A. Weinberg. 2011. Hallmarks of cancer: the next generation. *Cell*. 144:646–674. http://dx.doi.org/10.1016/j.cell.2011.02.013

Hannedouche, S., J. Zhang, T. Yi, W. Shen, D. Nguyen, J.P. Pereira, D. Guerini, B.U. Baumgarten, S. Roggo, B. Wen, et al. 2011. Oxysterols direct immune cell migration via EBI2. *Nature*. 475:524–527. http://dx.doi.org/10.1038/nature10280

Hong, C., Y. Kidani, N. A-Gonzalez, T. Phung, A. Ito, X. Rong, K. Ericson, H. Mikkola, S.W. Beaven, L.S. Miller, et al. 2012. Coordinate regulation of neutrophil homeostasis by liver X receptors in mice. *J. Clin. Invest.* 122:337–347. http://dx.doi.org/10.1172/JCI58393

Horton, J.D., J.L. Goldstein, and M.S. Brown. 2002. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J. Clin. Invest.* 109:1125–1131.

Jensen, H.K., F. Donskov, N. Marcussen, M. Nordsmark, F. Lundbeck, and H. von der Maase. 2009. Presence of intratumoral neutrophils is an independent prognostic factor in localized renal cell carcinoma. *J. Clin. Oncol.* 27:4709–4717. http://dx.doi.org/10.1200/JCO.2008.18.9498

Liu, C., X.V. Yang, J. Wu, C. Kuei, N.S. Mani, L. Zhang, J. Yu, S.W. Sutton, N. Qin, H. Banie, et al. 2011. Oxysterols direct B-cell migration through EBI2. *Nature*. 475:519–523. http://dx.doi.org/10.1038/nature10226

Liu, S.Y., R. Aliyari, K. Chikere, G. Li, M.D. Marsden, J.K. Smith, O. Pernet, H. Guo, R. Nusbaum, J.A. Zack, et al. 2013. Interferon-inducible cholesterol-25-hydroxylase broadly inhibits viral entry by production of 25-hydroxycholesterol. *Immunity*. 38:92–105. http://dx.doi.org/10.1016/j.immuni.2012.11.005

Moore, K.J., and I. Tabas. 2011. Macrophages in the pathogenesis of atherosclerosis. *Cell*. 145:341–355. http://dx.doi.org/10.1016/j.cell.2011.04.005

Motz, G.T., and G. Coukos. 2011. The parallel lives of angiogenesis and immunosuppression: cancer and other tales. *Nat. Rev. Immunol.* 11:702–711. http://dx.doi.org/10.1038/nri3064

Russell, D.W. 2000. Oxysterol biosynthetic enzymes. *Biochim. Biophys. Acta*. 1529:126–135. http://dx.doi.org/10.1016/S1388-1981(00)00142-6

Saintigny, P., E. Massarelli, S. Lin, Y.H. Ahn, Y. Chen, S. Goswami, B. Erez, M.S. O'Reilly, D. Liu, J.J. Lee, et al. 2013. CXCR2 expression in tumor cells is a poor prognostic factor and promotes invasion and metastasis in lung adenocarcinoma. *Cancer Res.* 73:571–582. http://dx.doi.org/10.1158/0008-5472.CAN-12-0263

Singh, J.K., G. Farnie, N.J. Bundred, B.M. Simões, A. Shergill, G. Landberg, S.J. Howell, and R.B. Clarke. 2013. Targeting CXCR1/2 significantly reduces breast cancer stem cell activity and increases the efficacy of inhibiting HER2 via HER2-dependent and -independent mechanisms. *Clin. Cancer Res.* 19:643–656. http://dx.doi.org/10.1158/1078-0432.CCR-12-1063

Villablanca, E.J., L. Raccosta, D. Zhou, R. Fontana, D. Maggioni, A. Negro, F. Sanvito, M. Ponzoni, B. Valentinis, M. Bregni, et al. 2010. Tumor-mediated liver X receptor-alpha activation inhibits CC chemokine receptor-7 expression on dendritic cells and dampens antitumor responses. *Nat. Med.* 16:98–105. http://dx.doi.org/10.1038/nm.2074

Voet, D., and J.G. Voet. 2011. Biochemistry. John Wiley & Sons, Hoboken, NJ. 1453 pp.

Westerterp, M., A.J. Murphy, M. Wang, T.A. Pagler, Y. Vengrenyuk, M.S. Kappus, D.J. Gorman, P.R. Nagareddy, X. Zhu, S. Abramowicz, et al. 2013. Deficiency of ATP-binding cassette transporters A1 and G1 in macrophages increases inflammation and accelerates atherosclerosis in mice. *Circ. Res.* 112:1456–1465. http://dx.doi.org/10.1161/CIRCRESAHA.113.301086